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Using Bioinformatics Tools to Analyze the Effect of Steviol Glycosides on the C-Glucan Binding Protein of Bacteria Streptococcus mutans Compared to Eugenol

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Keywords: Bioinformatics Tool, Steviol Glycosides, Streptococcus Mutans, Eugenol, Docking Accuracy, Pyrx.

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Abstract:

Dental caries is a common and chronic condition caused by bacterial activity that erodes the tooth structure. *Streptococcus mutans* is the main causative bacteria, which adheres to tooth surfaces and breaks down carbohydrates to produce acids. These acids demineralize tooth enamel, leading to caries. In the current research, the focus was on eugenol and steviol glycosides, which have medicinal properties. Eugenol is a natural compound with analgesic, antibacterial, antiviral, and antioxidant properties, making it a promising option in caries prevention. Steviol glycosides are substances extracted from the stevia plant, and have a wide range of health benefits, including their ability to prevent caries, but their mechanism of action is still under investigation. The aim of the study was to evaluate the efficacy of steviol glycosides compared to eugenol in their interaction with GbpC protein in Streptococcus mutans, and to provide evidence for the potential use of steviol glycosides as a natural option for the prevention of dental caries. In this research, we used an in-silico approach to investigate complexes of 5UQZ (Glucan Binding Protein C) in chain A with eugenol as a control and Steviol glycosides. A virtual screening experiment was performed using PyRx software to improve the accuracy of the association between the compounds and the target protein. The

results showed that eugenol achieved a binding degree of -5.9 kcal/mol, while steviol glycosides recorded a higher binding degree of -9.8 kcal/mol. These results indicate that steviol glycosides have a higher efficacy in interacting with the target protein than eugenol. This research highlights the promising potential of steviol glycosides as a natural option for combating dental caries, and lays the foundation for future studies aimed at developing effective and sustainable oral health products.

Keywords: Bioinformatics Tool, Steviol Glycosides, *Streptococcus Mutans,* Eugenol, Docking Accuracy, PyRx.

استخدام أدوات المعلوماتية الحيوية لتحليل تأثير ستيفيول جليكوسيدات على بروتين ربط سي-جلوكان للبكتيريا العقدية الطافرة مقارنة بالأوجينول

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الخلاصة:

تسوس الأسنان هو حالة شائعة ومزمنة ناجمة عن نشاط بكتيري يؤدي إلى تآكل بنية الأسنان. تعد *Streptococcus mutans* هي البكتيريا المسببة الرئيسية، والتي تلتصق بأسطح الأسنان وتحلل الكربوهيدرات لإنتاج الأحماض. تعمل هذه الأحماض على إزالة المعادن من مينا الأسنان، مما يؤدي إلى تسوس الأسنان. يركز البحث الحالي على الأوجينول وجليكوسيدات ستيفيول، والتي لها خصائص طبية. الأوجينول هو مركب طبيعي له خصائص مسكنة ومضادة للبكتيريا ومضادة للفيروسات ومضادة للأكسدة، مما يجعله خيارًا واعدًا في الوقاية من تسوس الأسنان. جليكوسيدات ستيفيول هي مواد مستخرجة من نبات ستيفيا، ولها مجموعة واسعة من الفوائد الصحية، بما في ذلك قدرتها على منع تسوس الأسنان، ولكن آلية عملها لا تزال قيد التحقيق. هدفت الدراسة إلى تقييم فعالية جليكوسيدات ستيفيول مقارنة بالأوجينول في تفاعلهما مع بروتين GbpC في *Streptococcus mutans*، وتقديم دليل على الاستخدام المحتمل لجليكوسيدات ستيفيول كخيار طبيعي للوقاية من تسوس الأسنان. استخدم هذا البحث نهجًا حسابيًا للتحقيق في معقدات 5UQZ (بروتين رابط الجلوكان C) في السلسلة A مع الأوجينول كعنصر تحكم وجليكوسيدات ستيفيول. تم إجراء تجربة فحص افتراضية باستخدام برنامج PyRx لتحسين دقة الارتباط بين المركبات والبروتين المستهدف. أظهرت النتائج أن الأوجينول حقق درجة ارتباط ٥,٩- كيلو كالوري / مول، في حين سجلت جليكوسيدات ستيفيول درجة ارتباط أعلى٩,٨ - كيلو كالوري / مول. تشير هذه النتائج إلى أن جليكوسيدات ستيفيول لها فعالية أعلى في التفاعل مع البروتين المستهدف من الأوجينول. يسلط هذا البحث الضوء على الإمكانات الواعدة لجليكوسيدات ستيفيول كخيار طبيعي لمكافحة تسوس الأسنان، ويضع الأساس لدراسات مستقبلية تهدف إلى تطوير منتجات صحة الفم الفعالة والمستدامة.

الكلمات المفتاحية: أداة المعلوماتية الحيوية، جليكوسيدات ستيفيول، العقدية الطافرة، الأوجينول، دقة الالتحام، PyRx.

1. Introduction:

Dental caries is a major dental and public health hazard worldwide, particularly among low-income people. Frequent consumption of dietary carbohydrates, particularly sweets, raises the risk of dental caries by generating acidity and upsetting the symbiotic, diverse, and complex microbial population of the mouth [1, 2]. Excessive acid production alters the nature of the bacterial biofilm, demineralizes tooth structure, and produces cavities. Acidic and acid-tolerant bacteria that bind to cavities include Streptococcus mutans, Lactobacillus, Actinomyces, Bifidobacterium, and Scardovia. The microbiota composition varies based on the tooth site, severity of carious lesions, and the rate of disease progression [1]. *Streptococcus mutans*, a pathogenic bacterium, is regarded as the primary factor in dental caries; it is assumed to be the most cariogenic bacterium. When it comes in contact with sweet or sucrose-containing products, it multiplies and secretes acids and other substances that damage the enamel of teeth [3, 4]. *Streptococcus mutans*, a gram-positive, facultative anaerobic coccus (round bacterium) commonly found in the human oral cavity, is a primary cause of tooth decay [5]. S. mutans' natural habitat is the human oral cavity, specifically dental plaque, a multispecies biofilm that forms on the hard surfaces of teeth [6]. *Streptococcus mutans* has been identified as the major bacterium causing dental caries in people. The bacteria create numerous glucan-binding proteins (GBPs), one of which is Glucan Binding Protein C (GbpC). When stressed, GbpC, a member of the streptococcal protein family PAc, is expressed. [7]. *Streptococcus mutans* are large cariogenic organisms, the result of their ability to manufacture huge volumes of glucans and acid, exceeding the salivary buffering capacities, which gives the bacteria an advantage to outcompete noncariogenic commensal species at low pH circumstances [8].

Eugenol is an allyl-substituted guaiacol from the allylbenzene family. It is a colorless to pale yellow, aromatic, oily liquid made from essential oils, most notably clove, nutmeg, cinnamon, basil, and bay leaf. It is found in levels of 82-88% in clove leaf oil and 80-90% in clove bud oil [9]. Eugenol, also known as 4-allyl-2-methoxyphenol, is an aromatic chemical. It is an organic chemical compound belonging to the phenols category. Its chemical formula is $C_{10}H_{12}O_2$. It consists of a benzene ring linked to a methoxyl group ($-OCH_3$) and also to a hydroxyl group (OH). According to numerous studies, this molecule has analgesic, antiviral, antioxidant, antibacterial, and anti-inflammatory characteristics; it is also used in dentistry to treat pulpitis and toothaches [9]. Some research suggests that eugenol has an antimicrobial effect on S. mutans. Eugenol may be a useful therapeutic agent for dental caries since it has a better effect than the chemical Eugenol [10-12].

Stevia rebaudiana (Asteraceae) is a natural plant native to South America. Steviol glycosides are the chemical components that give the leaves their sweet taste [13]. Steviol glycosides show superior sweetening efficiency to sucrose and are calorie-free, non-cariogenic, and do not cause side effects [14]. Steviol is the main compound in stevioside, and it contains a structure consisting of 20 carbon atoms (C20H30O3), with a hydroxyl group (-OH) and a ring consisting of carbon. Stevia rebaudiana Bertoni has numerous health benefits, including antihyperglycemic, antihypertensive, antioxidant, renal protection, antitumor, antidiarrheal, diuretic, antiviral, immunomodulatory, and dental caries prevention [15]. Stevioside also helps reduce the risk of tooth decay, with some research showing it has anti-caries properties. However, more research is needed to discover how it works and the appropriate concentration of this substance [16-18].

2. Materials and methods:

2.1 Protein preparation and Ligands: *Streptococcus mutans'* Glucan Binding Protein C (GbpC), a primary target for Eugenol and Steviol glycosides, was acquired from the human PDB database using the PDB ID 5UQZ (URL: https://www.rcsb.org). Furthermore, the target proteins for the docking experiment are chosen based on their X-ray diffraction profiles. Proteins should be in PDB format. All heteroatoms, including ions and water, were removed from Streptococcus mutans' X-ray crystallographic structure in preparation for molecular docking. Chimera tool and Discovery Studio 2021 Client are used, and certain protein-binding sites in the chain are selected, while others are avoided.

2.2 Preparation of Ligands: Open Babel software was used to obtain SDF format for three-dimensional chemical structures, which were then translated to PDB format by Pubchem (https://pubchem.ncbi.nlm.nih.gov). Throughout the docking study, steviol glycosides were utilized as the reference molecule, with Eugenol serving as a control to assess correlation strength. Both of these compounds were obtained via PubChem.

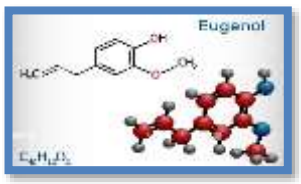
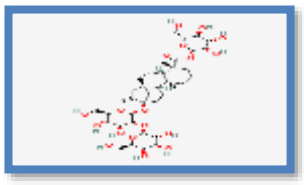
2.3 The process of docking molecules: The structural interactions between the (target protein) 5UQZ and the (ligand molecules) Steviol glycosides and Eugenol (ligand molecules (control)) have been investigated using an in-silico approach for analyzing ligand and receptor docking to display the conformation of this protein target selectivity. PyRx, a virtual screening tool, was used to increase docking accuracy by applying an algorithm as a scoring mechanism and integrating Vina and Auto Dock. [7] The target protein included 583 amino acid atoms. X-ray crystallography was used to precisely establish the chemical makeup of the macromolecule at 1.14 Å. The work continues with the UCSF Chimera software, which removes the bonds and

accompanying water molecules to prepare the target. In addition, all chains were eliminated except Chain A, which solely shows a ligand-binding site chain. Chain A was then added to the PyRx tool by marking it as a macromolecule in the PyRx workflow. Using auto dock techniques, the protein and ligand molecules were transformed into their suitable readable file format (pdbqt). All investigations of docking were carried out as blind docking in a grid box, which included all possible ligand-receptor complexes, and its dimensions were to Eugenol, as a control, and Steviol glycosides [(X = 100.54, Y =57.5798, and Z = 71.5732) and (X=92.5157, Y = 54.1111, and Z =67.8058)], respectively. All ligand bindings were allowed to freely rotate, and all other software parameters remained fixed while the receptor was considered as rigid. The final visualization of the docked structure was done using Discovery Studio Visualizer 3.0. Prior to assessing the efficiency of the compounds from Urtica dioica against 5UQZ [7].

3. Results

Molecular docking studies were carried out between the Glucan Binding Protein C (GbpC) of *Streptococcus mutans* (receptor) and Eugenol and steviol glycosides, as seen in **Table 1**. As the negative value of affinity binding increases, so does the precision of the receptor-ligand connection. The RMSD (Root Mean Square Deviation) number, which is used to confirm docking tests, represents the average difference between two proteins' corresponding atoms. [19].Each atom in one conformation is matched with itself in the opposite conformation by the RMSD upper bound, which ignores any symmetry. The RMSD lower bound is used to compare each atom in one conformation to the closest atom of the same element type in the other conformation [19].

Table 1: Diagnosis of ligands using Glucan Binding Protein C (GbpC) of *Streptococcus mutans*

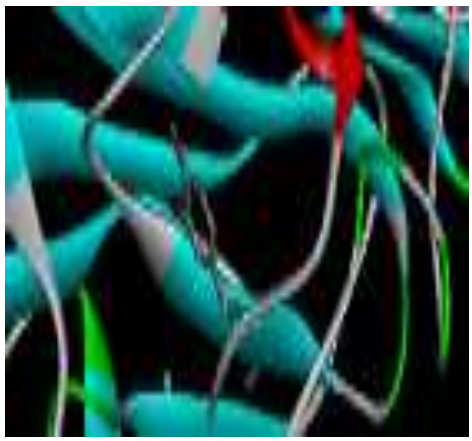
docking term for ligands	Binding Affinity kcal/mol	RMSD/UB	RMSD/LB	substance name
5uqz_3314uff_E= 169.59	-5.9	0.0	0.0 	Eugenol: 4-Allyl-2-methoxyphenol
5uqz_442089uff_ E=2828.21	-9.8	0.0	0.0 	Steviol glycosides

Eugenol: 4-Allyl-2-methoxyphenol as a Control Ligand

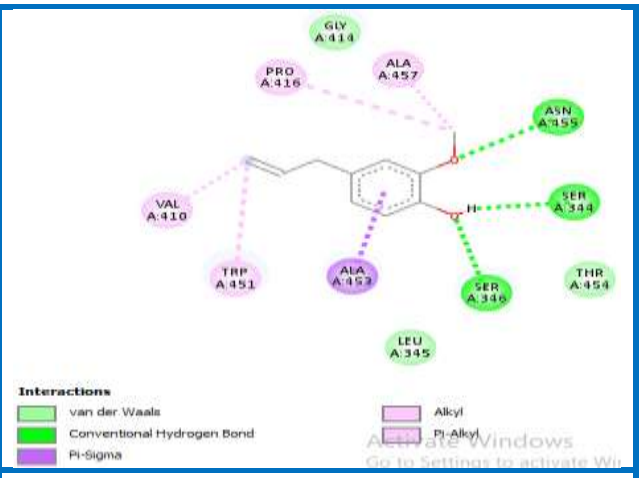
Eugenol (4-Allyl-2-methoxyphenol) was the standard molecule used in the docking analysis, and it was retrieved from PubChem using PubChem CID-3314. Eugenols serve as a control in this investigation. Figure 1 shows the interaction state of the experimental control ligand. **Table 2** depicts the interaction of the ligand with chain A of *Streptococcus mutans'* Glucan Binding Protein C (GbpC), which contains eleven amino acids, as well as the five different types of interactions formed between the ligand and receptor. Van der Waals, Carbon hydrogen bond, Alkyle, Pi-Alkyle, and Pi-Sigma score binding energy (-5.9) increased the affinity between the two chemicals, as the binding strength was good. Usually, docking experiments are verified using the RMSD value. Eugenol's docked ligand and the experimental ligand shared an acceptable RMSD of 0.0 angstrom. **Table 2** depicts the interaction of amino acids and the type of bonds between ligands for Eugenol and chain A of Streptococcus mutans' Glucan Binding Protein C (GbpC). PyRx tool was used to do virtual screening by docking in a target protein's active region. The findings of this study also revealed that Eugenol had a good interaction with the A chain of Glucan Binding Protein C receptors, as illustrated in **Figure 1**.

Table 2: Streptococcus mutans and Eugenol's glucan binding protein C (GbpC) with amino acid position inside chain A

Amino acids	Location inside the chain	Bonds Type
THR	454	Van der -Waals
LEU	345	
GLY	414	
ASN	455	Carbon-hydrogen bond
SER	344	
SER	346	
PRO	416	Alkyle
ALA	457	
VAL	410	Pi- Alkyle
TRP	451	
ALA	453	Pi-Sigma



(a) Interactions between Glucan Binding Protein C (GbpC) of *Streptococcus mutans* and Eugenol at the active site (A) 3D representation



(b) 2D representation. Van der Waals, Carbon-hydrogen bond, Alkyle, Pi- Alkyle and Pi-Sigma are shown in the figure

Figure 1

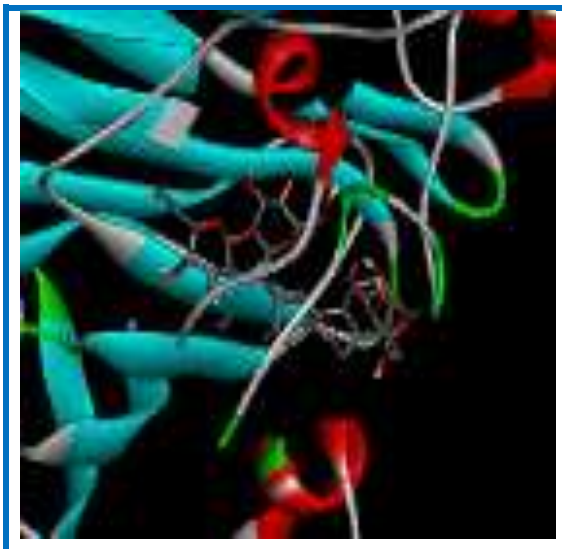
Steviol Glycosides

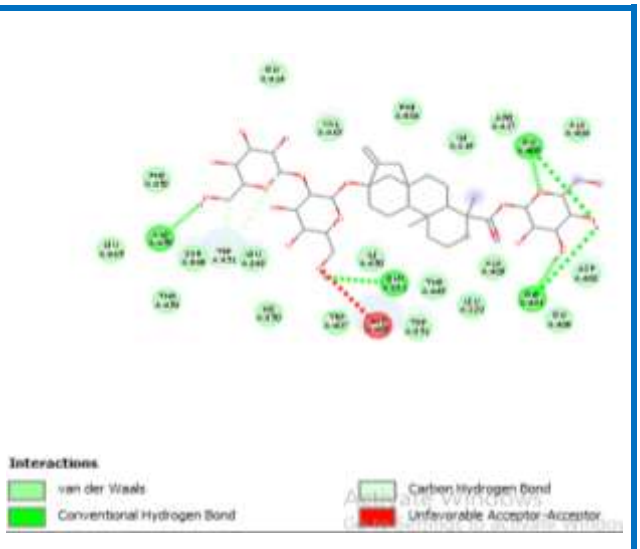
The ligand interacts with chain A of *Streptococcus mutans*. **Table 3** shows Glucan Binding Protein C (GbpC), which has twenty-two amino acids. There are four types of unfavorable acceptor-acceptor interactions: conventional hydrogen bonds, carbon-hydrogen bonds, and Van der Waals interactions between ligand and receptor. The binding energy (-9.8) improved the affinity between the two compounds since the binding strength was excellent. Typically, docking experiments are validated using RMSD values.

Steviol Glycosides docked with experimental ligands had RMSD differences of 0.0 angstrom, which was acceptable. The steviol glycosides and chain A of *Streptococcus mutans'* Glucan Binding Protein C (GbpC) were represented by amino acid interactions and the type of bonds between ligands, as shown in **Table 3**. The docking approach was employed with the PyRx program to perform a virtual screening of Steviol Glycosides in a target protein's active regions. The results of this investigation demonstrated that the bind had an excellent interaction with the A chain of the receptor *Streptococcus mutans* GbpC (Glucan Binding Protein C) with Steviol Glycosides, as shown in **Figure 2**.

Table 3: *Streptococcus mutans* Glucan Binding Protein C (GbpC) and Steviol Glycosides have amino acid locations within chain A.

Amino acids	Location inside the chain	Bonds Type
ASP	408	Unfavorable Acceptor-Acceptor
GLY	401	Conventional hydrogen bond
GLY	408	
GLN	391	
ALA	458	
TRP	451	Carbon-hydrogen bond
GLY	414	Van der- Waals
VAL	410	
PHE	304	
IEL	219	
APG	417	
ALA	404	
PHE	452	
LEU	345	
SER	346	
LEU	348	
THR	454	
HIS	350	
TRP	407	
ILE	450	
THR	449	
TRP	351	
LEU	220	
ALA	409	
ASP	402	
GLY	406	





(a) Interactions between Glucan Binding Protein C (GbpC) of Streptococcus mutans and Steviol Glycosides at the active site (A) 3D representation

(b) 2D representation. Unfavorable Acceptor-Acceptor, Conventional hydrogen bonds, carbon-hydrogen bonds, and Van der Waals are shown in the figure

Figure 2

4. Discussion

Eugenol (4-allyl-2-methoxyphenol) is the main component of the essential oil of this plant, and it is a natural compound with broad biological activity, as it has proven to be effective as an antibacterial and antifungal, including its effect on microbial biofilms. In addition, eugenol is non-toxic and safe for human use, making it a promising option in medical applications. Numerous studies have shown its ability to combat dental caries, and this is consistent with the results obtained, as the binding strength was good -5.9 kcal/mol **[20, 21,22]**, as this activity is linked to its strong interaction with caries-causing bacteria, which enhances its feasibility as a natural treatment.

On the other hand, the stevia plant stands out as a rich source of natural compounds with multiple health benefits, as studies indicate that it has anti-cancer and anti-inflammatory properties, in addition to its role in modifying the immune response and its diuretic effect. It is noteworthy that stevia also has a distinct role in oral and dental health, as its non-carbohydrate compounds prevent the growth of *Streptococcus mutans*, the bacteria associated with dental caries and cavity formation. Furthermore, research has shown that the use of Stevia rebaudiana Bertoni leaf extracts as an herbal mouthwash shows clear efficacy in preventing caries **[23]**. The alcoholic extract of stevia also shows strong antibacterial activity against caries, and this agrees with the results obtained, as the binding strength was excellent, -9.8 kcal/mol

Based on the available scientific results, steviol glycosides, the active ingredient in stevia, appear as a promising option with antimicrobial properties that are superior in some aspects to eugenol. Studies have shown a strong affinity of steviol glycosides for the target bacteria, which opens up prospects for their use as a potential alternative to eugenol in the future. However, in-depth research is still needed to understand the exact mechanism of action of these glycosides, as well as to evaluate their efficacy in clinical settings, with a focus on the effect of crude extracts to maximize the benefit **[24, 25]**.

5. Conclusions

According to the findings of this study, the degree of binding for eugenol was -5.9 kcal/mol, whereas the degree of binding for Steviol Glycosides was -9.8 kcal/mol. Some research has discovered that stevioside has anti-mutagenic qualities and may help reduce the incidence of tooth decay; this confirms the results obtained, as steviol glycosides have a higher binding strength than eugenol. However, additional research is needed to determine the mechanism of action and the appropriate concentration of this substance, and raw Steviol Glycosides extract is preferred.

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